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THE CALCIUM HYPOTHESIS OF BRAIN AGING AND NEURODEGENERATIVE DISORDERS: SIGNIFICANCE IN DIABETIC NEUROPATHY

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Summary

In this paper we discuss the possible role of disturbed neuronal calcium homeostasis in brain aging and diabetic neuropathy. Disturbances in the homeostasis of cytosolic calcium concentration have been implicated in the pathogenesis of various acute and chronic neurodegenerative disorders and in brain aging. Obviously, these disorders do not all share the same pathogenetic mechanisms. However, a number of the pathogenetic mechanisms involved have in common that they may ultimately cause loss of calcium homeostasis, leading to neuronal damage. By identifying the possible role of calcium, treatment strategies can be developed that may be effective in a variety of neurodegenerative disorders, despite differences in their pathogenesis. Our aim is to explore some of the similarities that exist between a number of processes that have been implicated in the pathogenesis of brain aging and diabetic neuropathy, including ischemia, oxidative stress and non-enzymatic protein glycosylation. Each of these factors might impair neuronal calcium homeostasis, and ultimately lead to neurodegenerative changes. By discussing the putative role of these specific factors in two apparently dissimilar disorders, such as brain aging and diabetic neuropathy, we obviously do not intend to suggest that their pathogenesis is one and the same. Instead, by examining the relative role of these factors in two different types of neurodegenerative disorders we would like to emphasize the importance of disturbances in cellular calcium homeostasis as a final common pathway in neuronal damage resulting from various noxious events.

Key Words: calcium, diabetic neuropathy, aging, neurodegeneration

The calcium hypothesis of brain aging and neurodegenerative disorders postulates that cellular mechanisms for maintaining the cellular homeostasis of cytosolic calcium concentration play a key role in aging, and that sustained changes in calcium homeostasis could provide the final common pathway for the neuropathological changes associated with brain aging and various neurodegenerative disorders (1,2). In this paper we will review the putative role of disturbed calcium homeostasis in the pathogenesis of diabetic neuropathy, one of the most common of neurodegenerative disorders in man. In particular, we will try to explore some of the similarities that exist between a number of processes that have been implicated in the pathogenesis of diabetic neuropathy and factors that have been implicated in brain aging. These factors include ischemia, oxidative stress and non-enzymatic protein glycosylation. Each of these factors might impair neuronal calcium homeostasis, and ultimately lead to neurodegenerative changes.

The role of calcium in neural plasticity and neurodegenerative disorders

Neuronal plasticity can be defined as the capacity of the neuron to adapt to a changing internal or external environment, to previous experience or to recover from trauma (3). Long-term maintenance of the integrity of the nervous system is proposed to be regulated by a functional equilibrium between plastic mechanisms promoting neuronal growth and regeneration and those processes that control regression and degeneration (2). Changes in neuronal plasticity may disrupt the balance between growth and regression, and thus shift the equilibrium in favour of degeneration. Neuronal plasticity is critically dependent on a fine regulation of $[Ca^{2+}]_i$ (4,5). Hence, alterations in neuronal calcium homeostasis may impair neuronal plasticity and thus contribute to the pathogenesis of neurodegenerative and age-related brain disorders.

Under resting conditions, the intracellular calcium concentration ($[Ca^{2+}]_i$) in neurons is about 0.1 μM, whereas the extracellular calcium concentration is about 1 mM. Activation of the neuron induces transient fluctuations of $[Ca^{2+}]_i$, thus mediating responses to environmental signals. The transient increase in $[Ca^{2+}]_i$ is dependent on calcium entry through voltage- or receptor-operated calcium channels and on release of calcium from intracellular stores. Thereafter, $[Ca^{2+}]_i$ is rapidly restored to basal levels by binding to calcium-binding proteins, sequestration into the endoplasmatic reticulum and the mitochondria, and extrusion from the cell by ATP-driven Ca^{2+} pumps and the Na^+/Ca^{2+}-exchanger (1). Modulation of $[Ca^{2+}]_i$ thus plays a key role in the regulation of many neuronal functions, includin (1,4).

Evidence for involvement of disturbed neuronal calcium homeostasis in the pathogenesis of neurodegenerative disorders is considerable, and rapidly increasing (1,5). An excessive rise in $[Ca^{2+}]_i$ may have several detrimental effects on neurons, including lipolysis, proteolysis, alterations in protein phosphorylation, loss of integrity of the cytoskeleton and, ultimately, cell death (1). Alterations in $[Ca^{2+}]_i$ can damage neurons in a time and concentration dependent manner (2). In acute neurological disorders, such as stroke and hypoglycemic coma, neuronal damage the result of acute uncontrolled elevations in $[Ca^{2+}]_i$ (1). In contrast, in chronic neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease, brain aging and, as we suggest herein, diabetic neuropathy neuronal damage may be the result of relatively small changes in calcium homeostasis, that are sustained over a prolonged period (5,6).

Calcium homeostasis and brain aging

Brain aging appears to be associated with slowly progressive alterations in the mechanisms of calcium homeostasis. A significant increase in resting $[Ca^{2+}]_i$ in hippocampal and cortical neurons can be observed in senescent rats, as compared to neonatal and adult animals (7). In addition, the amount of calbindin, a calcium binding protein declines with age, thus impairing the calcium buffering capacity of neurons (8). Further, in hippocampal neurons of aged rabbits and rats calcium-mediated membrane potentials and transmembrane calcium currents appear to be enhanced (9,10). The observed changes were most apparent in the calcium current through L-type voltage operated calcium channels, which appeared to be particularly affected by the aging process (10). Behavioural (e.g. water labyrinth learning, eye blink conditioning) and electrophysiological (e.g. frequency potentiation of excitatory pathways in the hippocampus) studies in aging-rats and rabbits provide evidence for age-related deficits in synaptic plasticity (9,10). This deficit in synaptic plasticity provides indirect information on $[Ca^{2+}]_i$ regulation, and was suggested to be related to the aforementioned increased calcium currents.

The hypothesis that L-type calcium channels may play a role in the age-related disturbances of $[Ca^{2+}]_i$ homeostasis and associated changes in neuronal plasticity has initiated attempts to prevent these disturbances with L-channel blockers. Nimodipine is a dihydropyridine class L-type calcium channel antagonist which is known for its cardiovascular effects. In addition, nimodipine binds to brain tissue and has marked neuropharmacological effects (11). Nimodipine affects calcium homeostasis in spinal cord neurons in culture (12) and protects against the effects of hypoxia (4) and oxidative stress (13) at the cellular level. In aging animals nimodipine was shown to ameliorate age-related deficits in learning and frequency potentiation of excitatory pathways in the hippocampus, indicating an improvement of synaptic plasticity (9,10,14).

Several factors that appear to be involved in brain-aging may directly, or indirectly, affect neuronal $[Ca^{2+}]_i$ homeostasis [For review (5,7,9)]. In the present paper we will briefly discuss those factors that have also been implicated in the pathogenesis of diabetic neuropathy, including i) increases in oxidative stress, ii) increases in non-enzymatic protein glycosylation and iii) ischemia. By discussing the putative role of these specific factors in aging as well as diabetes we obviously do not intend to suggest that the pathogenesis of brain aging and diabetic neuropathy are one and the same. Instead, by examining the relative role of these factors in two different types of neurodegenerative disorders we would like to emphasize the importance of disturbances in cellular calcium homeostasis as a final common pathway in neuronal damage resulting from various noxious events.

Oxidative stress, resulting from increased formation and/or reduced scavenging of reactive oxygen species (ROS), can damage critical biological molecules and initiate a cascade of events (15). Interestingly, increased ROS production and elevations of $[Ca^{2+}]_i$ appear to be connected (13,16); increased ROS formation leads to increases in $[Ca^{2+}]_i$ (13), and *vice versa* (16). An increased production of ROS, which was associated with an increased oxidation of proteins and lipids, has been demonstrated in the brains of aging rodents (17,18). Further, decreased activity of antioxidant enzymes and increased oxidation of proteins have been demonstrated in the brains of aging humans (19). Experimental studies have shown that antioxidant treatment can ameliorates the age-related changes in protein oxidation, as well as age-related cognitive decline (18).

Aging is also associated with an accumulation of advanced glycosylation end products (AGEs) in various tissues (20). AGEs are long-lived macromolecules which have been irreversibly modified by glucose, via the so-called Maillard reaction [Review (21)], as a function of glucose concentration and time. The increase in AGE content in aging might be related to a lower protein turnover (20). Interestingly, the formation of AGEs is associated with the increased production of ROS (20,21), thus linking the pathophysiological model of non-enzymatic glycosylation to the previously described mechanism of oxidative stress.

Finally, during aging, brain capillaries may undergo progressive degeneration caused by amyloid deposits, thickened basement membrane, and reduced vessel elasticity (22,23). In the long term, capillary abnormalities may lead to increased capillary resistance, which in turn can affect cerebral blood flow. Disturbed blood flow to the brain may impair the delivery of essential nutrients, particularly oxygen and glucose, to cerebral neurons, and thus compromise energy availability to neurons. Both AGEs and oxidative stress may be involved in the age-related vascular dysfunction (24,25). AGEs may quench the vasodilating compound nitric oxide (24). In addition, endothelial oxidative damage and endothelial dysfunction have been observed in the presence of AGEs (25). Possibly, disturbed $[Ca^{2+}]_i$ homeostasis plays a role in these vascular abnormalities as well. This is supported by the finding that nimodipine, in addition to its effects on neuronal calcium homeostasis, counteracted the age-related changes in the cerebral microvasculature (8).

Diabetic neuropathy

Peripheral neuropathy is a frequent complication of diabetes mellitus and a leading cause of polyneuropathy in the Western world. Several patterns of neuropathy can be distinguished, of which distal symmetric polyneuropathy is the most common (26,27). The typical symptoms of distal symmetric polyneuropathy, such as complaints of numbness, paraesthesia and a tingling or prickling feeling, are most pronounced in the lower limbs. Electrophysiological examination of neuropathic patients shows impairment of motor and sensory nerve conduction velocity and reductions in nerve action potential amplitude. Autonomic neuropathy is another common form of diabetic neuropathy, which leads to many significant problems such as postural hypotension, gastroparesis, diarrhoea, constipation, neurogenic bladder, and male impotence (28). At present, no effective clinical treatment for diabetic neuropathy is available, although several studies have demonstrated that intensive insulin therapy effectively delays its onset and slows its progression (29,30). In a proportion of diabetic patients however, neuropathy develops and progresses despite intensive insulin therapy (29).

The majority of studies on the pathogenesis and treatment of diabetic neuropathy has been performed in experimentally diabetic rats. Two rat models are used most frequently. Firstly, streptozotocin (STZ)-induced diabetes. STZ destroys pancreatic ß-cells relatively selectively (31), leading to insulin deficiency and hyperglycaemia. Secondly, the BB/Wor model, which involves spontaneously occurring diabetes secondary to an immune-mediated destruction of the ß-cells (32). Neuropathy in these two rat models mimics clinical diabetic neuropathy in several aspects. As in diabetic patients, early neurological dysfunction in both STZ- and BB/Wor diabetic rats is characterised by reductions in motor and sensory nerve conduction velocities (33). These early reductions in conduction velocity are mostly rapidly reversible (34). A further progressive impairment of nerve conduction is related to morphological changes in the nerve which are generally more persistent (35,36).

The pathogenesis of diabetic neuropathy

The pathogenesis of diabetic neuropathy is multifactorial. Several mechanisms may be involved, including metabolic changes (37), neurovascular dysfunction (38) and changes in trophic support (39).

Metabolic changes that may be involved in the pathogenesis of diabetic neuropathy include increased polyol pathway flux, increased oxidative stress and enhanced non-enzymatic protein glycosylation. Increased polyol pathway flux has long been considered the central mechanism in the pathogenesis of diabetic complications, among which neuropathy (37,40). In the polyol pathway excess glucose is converted to sorbitol which impairs phosphoinositide metabolism, and protein kinase C and Na^+,K^+-ATPase activity. The reduced Na^+,K^+-ATPase activity leads to intra-axonal Na^+ accumulation, which may link the increased polyol pathway flux to the nerve conduction abnormalities in diabetic neuropathy (37,40). Further, the intra-axonal Na^+ accumulation may modulate the activity of the transmembrane Na^+/Ca^{2+}-exchanger, thus affecting $[Ca^{2+}]_i$ homeostasis.

Diabetes is also associated with an increased production of ROS [Review (41)]. Increased ROS production in diabetes is a consequence of the process of glucose autooxidation. This process, in which glucose is oxidised in the presence of free metal ions, leads to superoxide and hydroxyl radical release, and finally causes protein oxidation. In addition, diabetes is associated with a reduction in ROS scavenging compounds, like glutathione, catalase and superoxide dismutase. In the sciatic nerve from STZ-diabetic rats, increased conjugated dienes and malonyldialdehyde as well as reduced superoxide dismutase and glutathione levels have been reported. Moreover, ROS

have been implicated in the development of vascular dysfunction in diabetes, and could also be responsible for scavenging of the vasodilating compound nitric oxide, and may thus be involved in nerve blood flow impairment (41). The efficacy of antioxidant drugs in the prevention and treatment of diabetic complications, among which neuropathy, has been widely tested. Various antioxidant drugs, i.e. GSH (42), probucol (43) and pharmacological doses of vitamin E (44) were shown to have a beneficial effect on diabetic neuropathy.

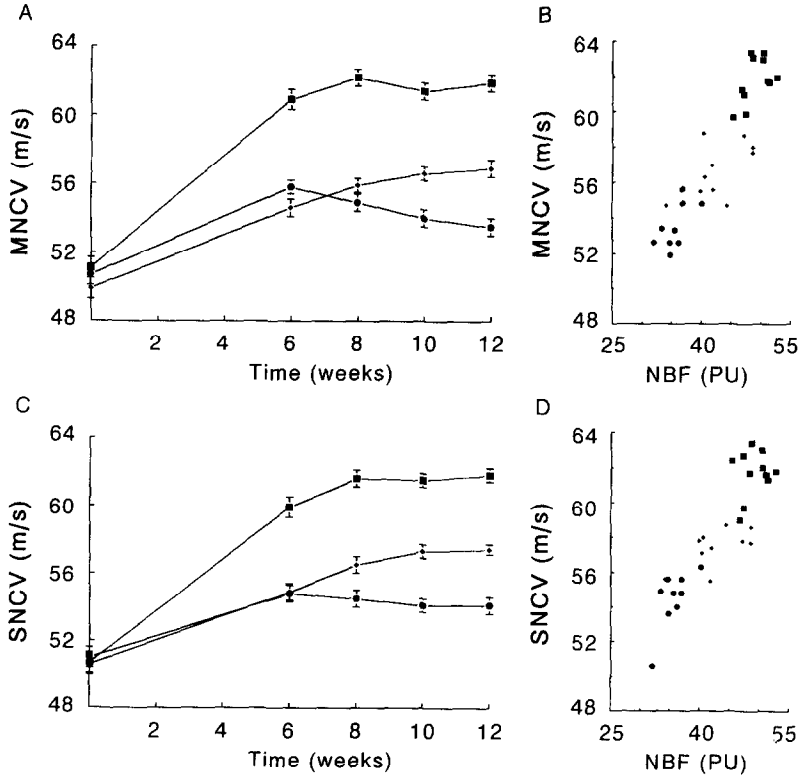

Fig. 1

A, C: Sciatic motor (A) and sensory (C) nerve conduction velocity (MNCV and SNCV *respectively*) in spontaneously diabetic BB/Wor rats; effect of nimodipine treatment. Treatment (20 mg/kg/48 hours i.p.) was initiated after six weeks of diabetes; ■: Non-diabetic control rats, ●: Untreated diabetic rats, ⋆: Nimodipine treated diabetic rats; data are mean ± SEM. MAVOVA: MNCV and SNCV were significantly impaired in untreated diabetic rats as compared to non-diabetic controls ($p<0.001$); nimodipine significantly ameliorated MNCV and SNCV in diabetic rats ($p<0.001$).

B, D: Correlation between sciatic nerve blood flow (NBF) in arbitrary perfusion units (PU), as determined with laser Doppler flowmetry, and MNCV (B) and SNCV (D) at week 12 of the experiment. Regression analysis NBF/MNCV: $R=0.81$, $p<0.001$; NBF/SNCV: $R=0.78$, $p<0.001$. Reprinted with permission from Kappelle *et al.* 1994 (52).

A third metabolic change in diabetes is an accelerated formation of AGEs. The presence of increased amounts of AGEs in tissues is generally associated with the development of diabetic complications, both by inducing structural changes to the extracellular matrix, as well as by modifying cell membrane and intracellular components [Review (45)]. Increased AGEs have been

reported in peripheral nervous tissue from diabetic animals (46,47). A direct causal relationship between AGEs in axons or myelin membrane structures and nerve dysfunction has not yet been established. However, non-enzymatic glycosylation of functionally important proteins like tubulin and Na-K-ATPase could be involved in, for example, impairment of axonal transport and nerve conduction (47,48).

In addition to these metabolic changes, it has become increasingly clear that reductions in the blood supply to the nerve play a critical role in the pathogenesis of diabetic neuropathy. In diabetic rats nerve blood flow, as well as endoneurial oxygen tension, are markedly reduced (49-51). Furthermore, amelioration of nerve blood flow by treatment with vasoactive agents correlates closely with improvement of nerve conduction velocity deficits of diabetic rats (Fig. 1) (52,53). Several studies in diabetic patients support the clinical relevance of these experimental findings. Endoneurial oxygen tension was shown to be reduced in neuropathic diabetic patients (54) and abnormalities of nerve blood flow were demonstrated with fluorescein angiography (55). Moreover, the extent of morphological endoneurial microvascular abnormalities was shown to be correlated to the severity of neuropathy (56). The exact mechanism of nerve blood flow impairment in diabetes is not known, but diabetes-related changes in the levels of circulating or locally produced vasoactive factors are likely to play a role: increases in plasma endothelin and angiotensin converting enzyme levels, impaired production or release of nitric oxide, and reduced prostacyclin synthesis have been reported [Review (38)].

The role of disturbed calcium homeostasis in the pathogenesis of diabetic neuropathy

Although, various metabolic and vascular abnormalities apparently are involved in the pathogenesis of diabetic neuropathy, the final pathway through which these metabolic and vascular derangements lead to nerve dysfunction is as yet not completely clear. We suggest that disturbed neuronal $[Ca^{2+}]_i$ homeostasis may well be involved, analogous with the proposed role for changes in $[Ca^{2+}]_i$ in brain aging and neurodegenerative disorders. Abnormal $[Ca^{2+}]_i$ regulation and calcium channel activity have been described in various diabetic tissues, including arteries, myocardium, skeletal muscle, erythrocytes and kidney [Review (57)], and have been implicated in the pathogenesis of secondary complications of diabetes in these tissues.

In peripheral nerve of diabetic rats mitochondrial and axoplasmic calcium levels were indeed found to be increased with electron-probe X-ray microanalysis (58). It should be noted however, that this technique cannot distinguish between free Ca^{2+} and calcium that was present in non-ionized form. Moreover, voltage-dependent calcium currents through L-, and N-channels are enhanced in dorsal root ganglion neurones of BB/Wor rats and diabetic mice *in vitro* (59,60). In contrast, the activity of the Na^+/Ca^{2+}-exchanger (40) and Ca^{2+}-ATPase (61) are impaired, leading to a net calcium overload. Again, studies on neuronal plasticity provide indirect evidence for diabetes-related changes in neuronal $[Ca^{2+}]_i$ regulation: peripheral nerve regeneration is impaired in diabetes (62), and diabetes may be associated with cognitive deficits (63).

In addition to the effects of changes in $[Ca^{2+}]_i$ on the neuron, disturbed $[Ca^{2+}]_i$ regulation may also affect the vasculature. Altered $[Ca^{2+}]_i$ regulation could thus be involved in nerve blood flow impairments. In the aorta of diabetic rats cytosolic free calcium was also found to be increased (64). This increase may be related to changes in the activity of vascular Ca^{2+}-ATPase and Na^+,K^+-ATPase (57,64). Moreover, transmembrane calcium currents in response to vasoactive compounds appear to be increased (65,66). Arteries of diabetic rats show enhanced contraction in response to activation of membrane calcium channels by the dihydropyridine class L-type calcium channel agonist Bay K 8644 (65,66). Further, nifedipine, a calcium channel antagonist directed to these

same calcium channels, reduced depolarisation induces contraction more potently in diabetic rats (66). The increased responsiveness of diabetic arteries to Bay K 8644 and nifedipine suggests that the number and/or activity of dihydropyridine sensitive calcium channels in arteries is increased in diabetes. Kappelle *et al.* have studied the effects of the dihydropyridine class L-type calcium channel antagonist nimodipine on experimental diabetic neuropathy extensively. Nimodipine was shown to improve nerve blood flow, as well as nerve conduction velocity (Fig. 1) (50,52). Improvement of nerve blood flow correlated closely with the amelioration of nerve conduction velocity (52), supporting the existence of a causal link between these two parameters. In a study by Robertson *et al.* nifedipine, a structurally related calcium channel blocker, was found to improve nerve conduction velocities in diabetic rats (67). Unfortunately nerve blood flow was not assessed in this particular study. In a preliminary study from our laboratory it was shown that nifedipine did improve both nerve blood flow and nerve conduction velocity, although to a lesser extent than nimodipine (Unpublished observations). Based on the aforementioned pathogenetic mechanisms, nimodipine may have a dual effect in diabetic neuropathy. Firstly, nimodipine may directly protect the neurones against the adverse effects of diabetes, by reducing calcium influx through neuronal L-channels. Secondly, by improving nerve blood flow, nimodipine may reduce endoneurial hypoxia, thus improving nerve function. The relative contribution of these neuronal and vascular effects of nimodipine remains to be determined.

Conclusion

Diabetic neuropathy is a frequent, troublesome complication of diabetes mellitus, for which no adequate clinical treatment is currently available. The pathogenesis is multifactorial, although vascular disturbances leading to reductions in nerve blood flow and endoneurial oxygen tension apparently play a central role. Interestingly, some of the pathogenetic factors involved in diabetic neuropathy, in particular ischemia, oxidative stress and non-enzymatic protein glycosylation, may also play a role in age-related neurodegeneration. Obviously, the relative contribution of these factors in the pathogenesis in these two neurodegenerative disorders differs. Moreover, degenerative changes appear to affect different subpopulations of neurons in aging and diabetes. Therefore, as already stated we do not suggest that the pathogenesis of neurodegenerative disorders in aging and diabetes are one and the same. However, what we have tried to point out in this paper is that the complex interplay of different noxious events, such as increased oxidative stress and ischemia, may ultimately lead to various neurodegenerative disorders via mechanisms that are at least in part related to disturbances of calcium homeostasis. Calcium channel blockers, such as nimodipine, may play a dual role in the treatment of these disorders: firstly through vascular effects, leading to improved blood flow, and secondly by ameliorating disturbed neuronal calcium homeostasis.

References

1. B.K. SIESJO, Ann.N.Y.Acad.Sci. 747 140-161 (1994).
2. Z.S. KHACHATURIAN, Ann.N.Y.Acad.Sci. 747 1-11 (1994).
3. W.H. GISPEN AND F.P. HAMERS, Ann.N.Y.Acad.Sci. 747 419-430 (1994).
4. P.R. BäR, J. TRABER, T. SCHUURMAN AND W.H. GISPEN, J. Neural. Transm. Suppl. 31 55-71 (1990).
5. M.P. MATTSON, Ann.N.Y.Acad.Sci. 747 50-76 (1994).
6. G.A. DE ERAUSQUIN, E. COSTA AND I. HANBAUER, Pharmacol. Rev. 46 467-482 (1994).
7. A. VERKHRATSKY, A. SHMIGOL, S. KIRISCHUK, N. PRONCHUK AND P. KOSTYUK, Ann.N.Y.Acad.Sci. 747 365-381 (1994).
8. P.G.M. LUITEN, G.I. DE-JONG AND T. SCHUURMAN, Ann.N.Y.Acad.Sci. 747 431-451 (1994).

9. J.F. DISTERHOFT, J.R. MOYER, JR. AND L.T. THOMPSON, Ann.N.Y.Acad.Sci. 747 382-406 (1994).
10. P.W. LANDFIELD, Ann.N.Y.Acad.Sci. 747 351-364 (1994).
11. A. SCRIABINE, T. SCHUURMAN AND J. TRABER, FASEB. J. 3 1799-1806 (1989).
12. P.R. BäR, G.H. RENKEMA, C.M. VERAART, E.M. HOL AND W.H. GISPEN, Cell. Calcium 14 293-299 (1993).
13. V.L. SHEEN, E.B. DREYER AND J.D. MACKLIS, NeuroReport 3 705-708 (1992).
14. T. SCHUURMAN AND J. TRABER, Ann.N.Y.Acad.Sci. 747 467-474 (1994).
15. C.W. OLANOW, Trends. Neurosci. 16 439-444 (1993).
16. J.A. DYKENS, J. Neurochem. 63 584-591 (1994).
17. M. SAWADA AND J.C. CARLSON, Mech. Ageing. Dev. 41 125-137 (1987).
18. J.M. CARNEY, P.E. STARKE REED, C.N. OLIVER, R.W. LANDUM, M.S. CHENG, J.F. WU AND R.A. FLOYD, Proc. Natl. Acad. Sci. U. S. A. 88 3633-3636 (1991).
19. C.D. SMITH, J.M. CARNEY, P.E. STARKE REED, C.N. OLIVER, E.R. STADTMAN, R.A. FLOYD AND W.R. MARKESBERY, Proc. Natl. Acad. Sci. U. S. A. 88 10540-10543 (1991).
20. M.A. SMITH, L.M. SAYRE, V.M. MONNIER AND G. PERRY, Trends. Neurosci. 18 172-176 (1995).
21. S.P. WOLFF, Z.Y. JIANG AND J.V. HUNT, Free Radic Biol Med 10 339-352 (1991).
22. R.N. KALARIA AND P. HEDERA, Neuroreport. 6 477-480 (1995).
23. J.C. DE LA TORRE AND T. MUSSIVAND, Neurol Res. 15 146-153 (1993).
24. R. BUCALA, K.J. TRACEY AND A. CERAMI, J Clin Invest 87 432-438 (1991).
25. J.L. WAUTIER, M.P. WAUTIER, A.M. SCHMIDT, G.M. ANDERSON, O. HORI, C. ZOUKOURIAN, L. CAPRON, O. CHAPPEY, S.D. YAN, J. BRETT AND ET AL, Proc. Natl. Acad. Sci. U. S. A. 91 7742-7746 (1994).
26. A.I. VINIK, M.T. HOLLAND, J.M. LE BEAU, F.J. LIUZZI, K.B. STANSBERRY AND L.B. COLEN, Diabetes Care 15 1926-1975 (1992).
27. M.A. ROSS, Med. Clin. North. Am. 77 111-124 (1993).
28. E. NIAKAN, Y. HARATI AND J.P. COMSTOCK, Metabolism. 35 224-234 (1986).
29. THE DIABETES CONTROL AND COMPLICATIONS STUDY GROUP, N. Engl. J. Med. 329 977-986 (1993).
30. K.F. AMTHOR, K. DAHL JORGENSEN, T.J. BERG, M.S. HEIER, L. SANDVIK, O. AAGENAES AND K.F. HANSSEN, Diabetologia. 37 579-584 (1994).
31. J.P. MORDES AND A.A. ROSSINI, Am. J. Med. 70 353-360 (1981).
32. L. CRISA, J.P. MORDES AND A.A. ROSSINI, Diabetes Metab. Rev. 8 9-37 (1992).
33. D.R. TOMLINSON, Diabetes Metab. Rev. 8 67-84 (1992).
34. P.O. JULU AND A. MUTAMBA, J. Neurol Sci. 106 56-59 (1991).
35. A.A. SIMA, S.A. LATTIMER, S. YAGIHASHI AND D.A. GREENE, J. Clin. Invest. 77 474-484 (1986).
36. S. YAGIHASHI, Diabetes Metab. Rev. 11 193-225 (1995).
37. D.A. GREENE, S.A. LATTIMER AND A.A. SIMA, N. Engl. J. Med. 316 599-606 (1987).
38. N.E. CAMERON AND M.A. COTTER, Diabetes Metab. Rev. 10 189-224 (1994).
39. W.J. BREWSTER, P. FERNYHOUGH, L.T. DIEMEL, L. MOHIUDDIN AND D.R. TOMLINSON, Trends Neurosci. 17 321-325 (1994).
40. D.A. GREENE, S. YAGIHASHI, S.A. LATTIMER AND A.A.F. SIMA, Am.J.Physiol. 247 E534-E539 (1984).
41. P.S. VAN DAM, B.S. VAN ASBECK, D.W. ERKELENS, J.J.M. MARX, W.H. GISPEN AND B. BRAVENBOER, Diabetes Metab. Rev. 11 181-192 (1995).
42. B. BRAVENBOER, A.C. KAPPELLE, F.P. HAMERS, T. VAN-BUREN, D.W. ERKELENS AND W.H. GISPEN, Diabetologia 35 813-817 (1992).
43. N.E. CAMERON, M.A. COTTER, V. ARCHIBALD, K.C. DINES AND E.K. MAXFIELD, Diabetologia. 37 449-459 (1994).

44. M.A. COTTER, A. LOVE, M.J. WATT, N.E. CAMERON AND K.C. DINES, Diabetologia 38 1285-1294 (1995).
45. M. BROWNLEE, Diabetes Care 15 1835-1843 (1992).
46. H. VLASSARA, M. BROWNLEE AND A. CERAMI, Proc Natl Acad Sci USA 78 5190-5192 (1981).
47. V.M. MONNIER, S. MIYATA, R.H. NAGARAJ AND D.R. SELL, Diabet Med 10 (suppl 2) 103S-106S (1993).
48. M.H. GARNER, A. BAHADOR AND G. SACHS, J. Biol. Chem. 26739 15058-15066 (1990).
49. E.J. STEVENS, A.L. CARRINGTON AND D.R. TOMLINSON, Diabetologia. 37 43-48 (1994).
50. A.C. KAPPELLE, G.J. BIESSELS, T. VAN BUREN, D.W. ERKELENS, D.J. DE WILDT AND W.H. GISPEN, Eur. J. Pharmacol. 250 43-49 (1993).
51. R.R. TUCK, J.D. SCHMELZER AND P.A. LOW, Brain 107 935-950 (1984).
52. A.C. KAPPELLE, G. BIESSELS, B. BRAVENBOER, T. VAN BUREN, J. TRABER, D.J. DE WILDT AND W.H. GISPEN, Br. J. Pharmacol. 111 887-893 (1994).
53. N.E. CAMERON, M.A. COTTER, K.C. DINES, E.K. MAXFIELD, F. CAREY AND D.J. MIRRLEES, Diabetologia 37 651-663 (1994).
54. P.G. NEWRICK, A.J. WILSON, J. JAKUBOWSKI, A.J. BOULTON AND J.D. WARD, Br. Med. J. Clin. Res. Ed. 293 1053-1054 (1986).
55. S. TESFAYE, N. HARRIS, J.J. JAKUBOWSKI, C. MODY, R.M. WILSON, I.G. RENNIE AND J.D. WARD, Diabetologia. 36 1266-1274 (1993).
56. S.T. BRITLAND, R.J. YOUNG, A.K. SHARMA AND B.F. CLARKE, Diabetes 39 909-913 (1990).
57. J. LEVY, J.R. GAVIN AND J.R. SOWERS, Am. J. Med 96 260-273 (1994).
58. J.M. LOWERY, J. EICHBERG, A.J. SAUBERMANN AND R.M.J. LOPACHIN, Diabetes 39 1498-1503 (1990).
59. E. KOSTYUK, N. PRONCHUK AND A. SHMIGOL, NeuroReport 6 1010-1012 (1995).
60. K.E. HALL, A.A.F. SIMA AND J.W. WILEY, J. Physiol. (Lond.) 486 313-322 (1995).
61. P.K. JANICKI, J.L. HORN, G. SINGH, W.T. FRANKS AND J.J. FRANKS, Life Sci. 55 PL359-PL364 (1994).
62. P.A. EKSTROM AND D.R. TOMLINSON, J. Neurol. Sci. 93 231-237 (1989).
63. G.J. BIESSELS, A.C. KAPPELLE, B. BRAVENBOER, D.W. ERKELENS AND W.H. GISPEN, Diabetologia 37 643-650 (1994).
64. T. OHARA, K.E. SUSSMAN AND B. DRANZNIN, Diabetes 40 1560-1563 (1991).
65. R.E. WHITE AND G.O. CARRIER, J. Pharmacol. Exp. Ther. 253 1057-1062 (1990).
66. B. TESFAMARIAM AND R.A. COHEN, Cardiovasc. Res. 29 549-554 (1995).
67. S. ROBERTSON, N.E. CAMERON AND M.A. COTTER, Diabetologia 35 1113-1117 (1992).